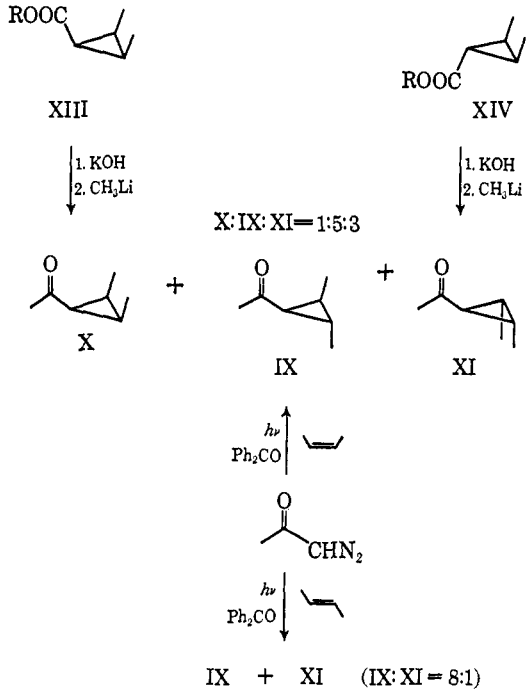
attributed to unfavorable interactions between the methyl groups and the proximate ring hydrogens.

The sensitized decomposition of diazoacetone in *cis*-2-butene leads to all three possible 1,2-dimethyl-3-acetylcyclopropanes. We have synthesized the two *cis* isomers X and XI from esters XIII and XIV of known[11] stereochemistry and have confirmed our and previous[11] assignments by noting that only XI and XIV are not rearranged on heating to 250°. The methyl–hydrogen interaction noted above is missing in these compounds, and it is the *anti,cis* isomer XI that predominates. We were unable to detect any of the least favored *syn,cis* isomer X in the products of reaction with *trans*-2-butene.



IX + XI (IX:XI = 8:1)

It is tempting to describe these changes in the most simple terms: that is, singlet carbenes are involved in the unsensitized and triplet in the sensitized decompositions. The singlet finds an accessible pair of electrons in the adjacent carbon–carbon single bond and reacts with these at a faster rate than with the more distant π electrons of the external olefin. Were the triplet to follow the same reaction path it would have to rearrange to a triplet ketene, many kilocalories/mole in energy above the ground-state singlet. The difficulty of this reaction allows the intermediate to add to the external π system of the solvent. The loss of stereochemical integrity is consistent with what has been observed for postulated triplet carbenes in solution.[12–14] Such an explanation, however comforting, is not necessarily correct. There is no evidence that in these reactions (or in many others) nitrogen has left the molecule at the time of reaction. The question of even the gross mechanism is therefore still open.

It is hoped that the control over product formation demonstrated in the preceding examples will prove useful in synthesis and be capable of extension to other intramolecular reactions of carbenes.

(11) W. von E. Doering and T. Mole, *Tetrahedron*, **10**, 65 (1960).
(12) K. R. Kopecky, G. S. Hammond, and P. A. Leermakers, *J. Am. Chem. Soc.*, **84**, 1015 (1962).
(13) M. Jones, Jr., W. Ando, and A. Kulczycki, Jr., *Tetrahedron Letters*, 1391 (1967).
(14) M. Jones, Jr., and K. R. Rettig, *J. Am. Chem. Soc.*, **87**, 4013, 4015 (1965), and references therein.

(15) Alfred P. Sloan Research Fellow, 1967–1968.

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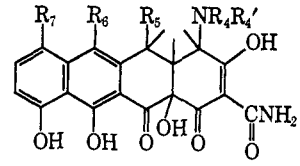
Biosynthesis of the Tetracyclines. IX. 4-Aminodedimethylaminoanhydrodemethylchlortetracycline from a Mutant of *Streptomyces aureofaciens*[1]

Sir:

At an early point in our work on the biosynthesis of the tetracycline antibiotics, we observed that the anhydrotetracyclines, **1**, were biologically reconverted to



1 (anhydrotetracyclines), $R_4 = R_4' = CH_3$; R_5 = H or OH; R_6 = H or CH_3; R_7 = H or Cl
2 (4-aminodedimethylaminoanhydrodemethylchlortetracycline), $R_4 = R_4' = R_5 = R_6 = H$; R_7 = Cl

the tetracyclines from which they were derived. The conversions were accomplished by the action of the tetracycline-producing species of the genus *Streptomyces*.[2] This observation was interpreted to mean that the anhydrotetracyclines were normal biosynthetic intermediates to the tetracyclines, and this concept successfully pointed the way to the identification of yet earlier stages in the biosynthetic pathway.[3]

Nonetheless, our failure to find any anhydrotetracycline-accumulating mutants of *S. aureofaciens* left some question that the original interpretation—fruitful though it was—might not be correct. The failure to find a mutant producing an anhydrotetracycline was finally rationalized by the thought that, since the anhydrotetracyclines are toxic to *Streptomyces*,[4] a mutant blocked at the 6-hydroxylation step might be self-lethal by virtue of accumulating the toxic anhydrotetracycline derivative and thus would not be expressed as a viable clone.

In 1964, Miller and coworkers[5] reported that inhibitors of biological methylation, such as ethionine, when added to tetracycline-producing fermentations resulted in the accumulation of N-demethyl analogs of the anhydrotetracyclines. This was felt to be good confirmation of the role of anhydrotetracyclines as biosynthetic intermediates. Meanwhile, we continued to search for a mutant of *S. aureofaciens* that would accumulate an anhydrotetracycline, and we now wish to report that mutant 1E1407[6] accumulates, as a major

(1) Paper VIII: J. R. D. McCormick and E. R. Jensen, *J. Am. Chem. Soc.*, **87**, 1794 (1965).
(2) J. R. D. McCormick, P. A. Miller, S. Johnson, N. Arnold, and N. O. Sjolander, *ibid.*, **84**, 3023 (1962).
(3) J. R. D. McCormick, U. H. Joachim, E. R. Jensen, S. Johnson, and N. O. Sjolander, *ibid.*, **87**, 1793 (1965).
(4) J. J. Goodman, M. Matrishin, and E. J. Backus, *J. Bacteriol.*, **69**, 70 (1955).
(5) P. A. Miller, A. Saturnelli, J. H. Martin, L. A. Mitscher, and N. Bohonos, *Biochem. Biophys. Res. Commun.*, **16**, 285 (1964).
(6) Mutant 1E1407 was isolated by Mr. N. Deduck and Dr. J. Growich of these laboratories as a nonproducing mutant of a demethylchlortetracycline-producing parent.

metabolite, 4-aminodedimethylaminoanhydrodemethylchlortetracycline[7] (**2**). This mutant of a 6-demethylchlortetracycline (DMCT) producing parental strain was observed to accumulate very little antibiotic (less than 2 μg/ml as DMCT). In mixed fermentations of 1E1407 with several other point-blocked mutants of *S. aureofaciens*, significant quantities of tetracycline antibiotics were produced by cosynthesis,[8] suggesting that 1E1407 was also point blocked in the biosynthetic pathway to the tetracyclines. The nature of the antibiotic accumulated in each instance was indicative of the relative locations of the blocks in 1E1407 and its cosynthesizing partner, as we have observed that a cosynthetic response is usually due to transfer of a partially finished tetracycline molecule from a donor to an acceptor cell. Thus a positive result usually has been observed only in transfer of an intermediate from the mutant having the later block to the one having the earlier block.[9] The positive results in these experiments led to testing a killed preparation of 1E1407 mash by addition to living cultures of other mutants, and now evidence of accumulation of a stable precursor (or precursors) by 1E1407 was found. In this situation, antibiotic is produced only when the precursor is a stable substance and occupies a place in the biosynthetic chain which is later than the point at which the test culture is blocked.

Absorption spectra of an acidic aqueous extract of 1E1407 fermented mash suggested the presence of an anhydrotetracycline-like substance, and this, together with the biological conversion data mentioned above, strongly suggested that the active precursor might be **2**.

Isolation of the precursor was accomplished by an adaptation of the method of Miller, *et al.*,[5] in which ethyl acetate extraction of the perchloric acid acidified whole mash was followed by simple partition of the crude material between chloroform and 0.1 *N* hydrochloric acid. The aqueous phase from the partition was evaporated to dryness to yield a partly crystalline crude product which was about 60% pure. Recrystallization was accomplished by dissolving the crude product in ten parts of 2 *N* hydrochloric acid in methoxyethanol and precipitating with toluene to give the pure product in good yield; absorption spectrum, λ_{max} mμ (ϵ): 424 (8600), 329 (3520), 314 sh (3720), 302 sh (5470), 269 (53,400), 223 (35,000); R_f 0.39 in butanol–0.1 *M* EDTA, pH 4.9, and 0.13 in butanol–0.1 *M* EDTA, pH 6.0. *Anal.* Found for $C_{19}H_{15}N_2O_7Cl_2 \cdot H_2O$: C, 48.45; H, 3.50; N, 5.85; H_2O, 4.06.

Biological conversion of **2** to DMCT was demonstrated in the usual way[2] utilizing *S. aureofaciens* mutant V828. A 34% conversion was found based on microbiological assay; the product, DMCT, was identified by paper chromatographic comparison with authentic material in two chromatographic systems. The pure substance, **2**, was shown by direct spectrophotometric and paper chromatographic comparison to be identical with the principal component in the partially purified material[10] reported by Miller, *et al.*[5]

This isolation of an anhydrotetracycline derivative from a mutant of *S. aureofaciens* affirms the earlier presumed role of the anhydrotetracyclines as intermediates in the biosynthetic pathway to the tetracyclines and reenforces the conclusions of Miller, *et al.*,[5] that the anhydrotetracyclines themselves arise by way of N-methylation of their amino analogs.[11]

(7) This compound has been previously described in terms of its chromatographic behavior and some chemical and biochemical properties.[5]

(8) J. R. D. McCormick, U. Hirsch, N. O. Sjolander, and A. P. Doerschuk, *J. Am. Chem. Soc.*, **82**, 5006 (1960).

(9) CF-1, a transferable hydrogenation cofactor, is the one exception to this. See P. A. Miller, N. O. Sjolander, S. Nalesnyk, N. Arnold, S. Johnson, A. P. Doerschuk, and J. R. D. McCormick, *ibid.*, **82**, 5002 (1960).

(10) A comparison sample of this material was kindly supplied by Dr. L. A. Mitscher of these laboratories.

(11) The earlier conclusion of one of us (J. R. D. M.) that N-methylation preceded reduction at C-4 has since been found to have been based on an isolation artifact at a key point. (See J. R. D. McCormick in "Antibiotics, Vol. 2, Biogenesis," D. Gottlieb and P. D. Shaw, Ed., Springer-Verlag, Berlin-Heidelberg, 1967.)

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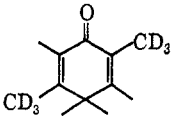
Additions and Corrections

Divinyl Sulfide: Copolymerization and Spectra [*J. Am. Chem. Soc.*, **81**, 2672 (1959)]. By CHARLES E. SCOTT and CHARLES C. PRICE, Chemistry Departments, University of Notre Dame, Notre Dame, Indiana, and University of Pennsylvania, Philadelphia, Pennsylvania.

In Table IV, change the values for ϵ_{max} for divinyl sulfide from 41,800 and 38,000 to 8350 and 7600.

Intermediates in the Photochemical Rearrangements of Bicyclo[3.1.0]hexenones [*J. Am. Chem. Soc.*, **89**, 1874 (1967)]. By HAROLD HART and DAVID W. SWATTON, Department of Chemistry, Michigan State University, East Lansing, Michigan 48823.

On page 1876, formula **11** should be



Acylation of Cyclooctatetraene Dianion and the Chemistry of Its Products [*J. Am. Chem. Soc.*, **89**, 5868 (1967)]. By THOMAS S. CANTRELL and HAROLD SHECHTER, Department of Chemistry, The Ohio State University, Columbus, Ohio 43210.